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Deciphering the Function of the Outer Membrane Protein OprD Homologue of *Acinetobacter baumannii*

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The increasing number of carbapenem-resistant *Acinetobacter baumannii* isolates is a major cause for concern which restricts therapeutic options to treat severe infections caused by this emerging pathogen. To identify the molecular mechanisms involved in carbapenem resistance, we studied the contribution of an outer membrane protein homologue of the *Pseudomonas aeruginosa* OprD porin. Suspected to be the preferred pathway of carbapenems in *A. baumannii*, the *oprD* homologue gene was inactivated in strain ATCC 17978. Comparison of wild-type and mutant strains did not confirm the expected increased resistance to any antibiotic tested. OprD homologue sequence analysis revealed that this protein actually belongs to an OprD subgroup but is closer to the *P. aeruginosa* OprQ protein, with which it could share some functions, e.g., allowing bacterial survival under low-iron or -magnesium growth conditions or under poor oxygenation. We thus overexpressed and purified a recombinant OprD homologue protein to further examine its functional properties. As a specific channel, this porin presented rather low single-channel conductance, i.e., 28 pS in 1 M KCl, and was partially closed by micro- and millimolar concentrations of Fe^{3+} and Mg^{2+}, respectively, but not by imipenem and meropenem or basic amino acids. The *A. baumannii* OprD homologue is likely not involved in the carbapenem resistance mechanism, but as an OprQ-like protein, it could contribute to the adaptation of this bacterium to magnesium- and/or iron-depleted environments.

Members of the genus *Acinetobacter* are strictly aerobic Gram-negative bacteria that are considered ubiquitous organisms widely distributed in nature. Their best-studied representative is the nosocomial pathogen *Acinetobacter baumannii*, which emerged in the 1970s and whose epidemic spread is now worldwide. This species causes a wide range of infections, including pneumonia and bloodstream infections, with a high crude mortality rate in intensive care units (4, 41). Two main factors account for the emergence of this problematic bacterial agent: its ability to persist in the hospital environment and its remarkable capacity to acquire mechanisms that confer resistance to antimicrobial agents. Multidrug-resistant (MDR) strains are spreading rapidly. A major cause for concern is their increasing resistance to all available drugs, especially carbapenems (11, 15, 44, 45), which are the most-used antibiotics for the treatment of severe infections caused by *A. baumannii* (15).

Carbapenem resistance in *A. baumannii* is achieved by classical mechanisms that occur in other bacterial species. Enzymatic inactivation via the production of carbapenem-hydrolyzing β-lactamases is the most common and well-documented mechanism (7, 13, 24, 46, 62). Besides, nonenzymatic mechanisms, like modifications of antibiotic membrane permeability, usually act in synergy with these enzymes to confer a high-level resistance phenotype (18, 20, 53, 60). These permeability modifications could be to the overexpression of multidrug RND efflux pumps (12, 33, 34, 47, 48, 50, 57) and/or modifications of outer membrane protein (OMP) expression or structure (9, 29, 30, 39, 40, 42, 54).

To date, three porins whose expression was shown to be reduced in several carbapenem-resistant strains may facilitate carbapenem diffusion through the *A. baumannii* outer membrane (OM): the carbapenem-associated OMP CarO (9, 29, 30, 39, 40), Omp33/36 (14), and a 43-kDa porin also called OprD homologue (8, 16, 19, 33). Among these proteins, the *A. baumannii* OprD homologue is an attractive candidate for the formation of carbapenem-specific channels since it displays 49% similarity to *Pseudomonas aeruginosa* OprD (16).

OprD is the prototype of a specific channel superfamily remarkable for its 19 members in *P. aeruginosa*. These proteins showed 46 to 57% similarity and played a significant role in the uptake of amino acids or organic acids, whether they fell into the OprD or the OpdK phylogenetic subgroup, respectively (58). OprD has been extensively studied on the basis of its structure, function, and involvement in *P. aeruginosa* carbapenem resistance (32). This 18-β-stranded barrel forms a very narrow channel (6) with two structural characteristics that might contribute to its channel specificity. A positively charged basic ladder provides an electrophoretic path for negatively charged compounds along the channel, whereas a negatively charged pocket located at the periplasmic end attracts positive substrates (6). According to this structure, this porin was demonstrated to promote the uptake of basic amino acids (arginine, histidine, lysine, and ornithine),

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small peptides containing these amino acids, and carbapenems by structural homology (23, 59). As a preferred portal of entry for these antibiotics, OprD is significantly involved in *P. aeruginosa* susceptibility to carbapenems. Thus, its loss from the OM can induce decreases in imipenem and meropenem susceptibility of 4- to 16-fold and 4- to 32-fold, respectively (26, 51).

However, few data are available on the functions of the OprD homologue in *A. baumannii* and its involvement in carbapenem resistance has not been demonstrated (16, 19). In the present study, we characterized this *A. baumannii* channel for the first time by *in vivo* and *in vitro* experiments. We provide evidence that this protein is not involved in carbapenem resistance and that its function is close to that of the *P. aeruginosa* OprQ porin.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *A. baumannii* ATCC 19606 was purchased from the Pasteur Institute Collection, Paris, France. *Escherichia coli* BL21λ(DE3)pLysS cells were used for the cloning and expression of the recombinant OprD (rOprD) homologue protein. All strains were stored in Luria-Bertani (LB) broth (BD Difco, France) containing 10% glycerol (Sigma-Aldrich, France) at −80°C. They were grown at 37°C in LB broth or LB agar medium supplemented with kanamycin (50 μg/ml; Sigma-Aldrich, France) to select mutant *A. baumannii* or with ampicillin (100 μg/ml; Sigma-Aldrich, France) and chloramphenicol (50 μg/ml; Sigma-Aldrich, France) for the *E. coli* transformant strain. For reverse transcription (RT)-PCR experiments, bacteria were grown overnight in M9 medium (Sigma-Aldrich, France) (37) containing 0.4% succinate as a carbon source and supplemented with 2 or 100 μM $FeCl_3$ or in LB broth under aerobic or oxygen-limited conditions. Bacteria were grown in 3 ml (aerobic condition) or 10 ml (microaerobic condition) of LB broth in 15-ml glass tubes to reduce the proportion of air in the glass tube compared to that of the medium.

Construction of *A. baumannii* Δ*oprD* homologue strain and MIC determination. Plasmid insertion into the *oprD*-like gene (A1S_0201 of *A. baumannii* ATCC 17978; GenBank gene identification no. 4917423) was performed as previously described (25), with slight modifications. Briefly, kanamycin and zeocin resistance plasmid pCR-Blunt II-TOPO (Invitrogen), which is unable to replicate in *A. baumannii*, was used as a suicide vector. An internal fragment (875 bp) of the *oprD*-like gene was amplified by PCR with primers OprDintFW (5′-TACTCCTGGTATTGT TGG) and OprDintRV (5′-CATCAGCACCATTAGATG) with genomic DNA from *A. baumannii* ATCC 17978 as the template. The PCR product was cloned into the pCR-BluntII-TOPO vector and electroporated into *E. coli* to yield the pTOPO-OprDint plasmid. The recombinant plasmid (0.1 μg) was then introduced into kanamycin- and zeocin-susceptible *A. baumannii* ATCC 17978 by electroporation as previously described (1). Mutants were selected on kanamycin-containing plates. Inactivation of the *oprD*-like gene by insertion of the plasmid via single-crossover recombination was confirmed by sequencing the amplified PCR product with primers T7 (5′-AATACGACTCACTATAGGG) and OprDextFW (5′-GT AACAATATAGAGTGAG). The MICs of the antibiotics tested (imipenem, meropenem, colistin, ceftazidime, and ciprofloxacin) were determined by Etest (AB Biodisk) in accordance with the manufacturer's instructions.

Semiquantitative RT-PCR. Extraction of RNAs was performed as previously described by Guyard-Nicodème et al. (21). Synthesis of cDNAs and PCR amplification of the *oprD* gene were achieved with 50 ng of total RNAs by using the Transcriptor One-Step RT-PCR kit (Roche, Mannheim, Germany) with the forward primer 5′-ATCGTAAGCTGAACCAT CGTT-3′ and the reverse primer 5′-TCATTTCTGCGGCAATAATTT-3′ and following the manufacturer's instructions. Synthesis of cDNAs and PCR amplification of the 16S RNA gene were done by using primers 5′-AAGCAACGCGAAGAACCTTA-3′ and 5′-CCGGACTACGATCGGTT TTA-3′ and a standard procedure, i.e., 5 min of denaturation at 94°C followed by 30 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 60°C, and 30 s of elongation at 72°C and a final elongation step of 10 min at 72°C. The amplified PCR fragments were then visually analyzed on 1.5% agarose gels. Experiments were repeated at least three times.

***E. coli* transformant strain for OprD homologue expression.** The 1,248-bp *oprD* homologue gene fragment lacking the code for the presumed amino-terminal signal sequence, with appropriate sites at both ends, was synthesized by PCR amplification. We used *A. baumannii* ATCC 19606 genomic DNA as the template with primers #261 (5′-TAT GGATCCAGCGAGCAAAGTGAGGCA AAAG-3′) and #262 (5′-TATAA GCTTTTAGAATAATTTCACAGGAATATC-3′). This DNA fragment was restricted with BamHI and HindIII and ligated into vector pETSIG. The latter is a pET derivative allowing the expression of the His-tagged protein of interest in the N-terminal fusion with the signal peptide of the *E. coli* OmpA porin to target the proteins to the membrane.

Expression, extraction, and purification of rOprD homologue. The expression of the OprD homologue protein in the *E. coli* transformant harboring the pETSIG derivative was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma-Aldrich, France) at an optical density at 600 nm of 0.4 to 0.5 (determined with a Cary 100 Bio spectrophotometer [Varian, Australia]). Growth was allowed to continue for an additional 2.5 h at 37°C with shaking at 140 rpm. The membrane pellet was obtained as described previously (54). We then used an extraction method previously described by Hamzehpour et al. (22) for *P. aeruginosa* OMP extraction in order to separate the inner membrane (IM) from the OM fractions. Briefly, the pellet was resuspended in 150 mM NaCl–20 mM Tris-HCl, pH 8, supplemented with 0.3% *N*-lauroylsarcosine (Sigma-Aldrich, France) and incubated for 30 min at room temperature. The OMP fraction was recovered in the pellet after centrifugation at 100,000 × *g* for 45 min at 18°C (Optima L-90K Ultracentrifuge; Beckman Coulter), and the IM protein fraction remained in the supernatant. Solubilization of the OprD homologue was then performed by vortexing the OM fraction for 1 h at room temperature with 0.3% *N*,*N*-dimethyldodecylamine *N*-oxide (LDAO; Sigma-Aldrich, France) buffered with 150 mM NaCl–20 mM Tris-HCl, pH 8. The suspension was centrifuged at 100,000 × *g* for 45 min at 18°C, and the rOprD homologue was recovered in the supernatant.

The presence of a His tag upstream of the OprD homologue protein allowed purification as previously described by Siroy et al. (54). In a final step, the hexahistidine (His_6) tag was cleaved by using the thrombin protease (GE Healthcare, France) to obtain the rOprD homologue free of the tag and ready for functional analyses (9). Conformation of the rOprD homologue protein was monitored by circular dichroism spectroscopy (CD6 spectropolarimeter; Jobin-Yvon, France) as described by Siroy et al. (54). Analysis by sodium dodecyl sulfate (SDS)-(polyacrylamide gel electrophoresis (PAGE) was performed using a 7 to 12% discontinuous gel system (dual vertical MGV-202; CBS Scientific Co.).

Reconstitution in planar lipid bilayers. For reconstitution experiments, virtually solvent-free planar lipid bilayers were formed by the technique of Montal and Mueller (38) as described by Siroy et al. (54). Briefly, the membrane was formed over a 150-μm hole in Teflon film (10 μm thick and treated with a mixture of 1:40 [vol/vol] hexadecane-hexane) separating two glass half cells. Current fluctuations were recorded with a BLM 120 amplifier (Biologic, Claix, France) and stored on a digital tape recorder (DTR 1202; Biologic, Claix, France). We used diphytanoylphosphatidylcholine (DPhPC; Avanti Polar Lipids) as lipids and 1 M KCl–20 mM HEPES (pH 7.4) as an electrolyte. The bulk concentrations of the reconstituted protein ranged from 10^{-9} to 10^{-8} M, depending on the single-channel or macroscopic measurements. Macroscopic conductance inhibition experiments were realized as previously described by Catel-Ferreira et al. (9). Substrate specificity was tested using imipenem (4 mM; Merck Sharp & Dohme-Chibret, Paris, France), meropenem (4 mM; VWR International, France), L-arginine (20 mM; Acros Organics), L-histidine (20 mM; Acros Organics), L-ornithine (20 mM; Merck, Germany), L-glutamic acid (20 mM; Sigma Chemical Company), or metallic ions like Fe^{3+} (5 to 100 μM), Al^{3+} (20 mM), and Mg^{2+} (2 to 40 mM; Sigma-

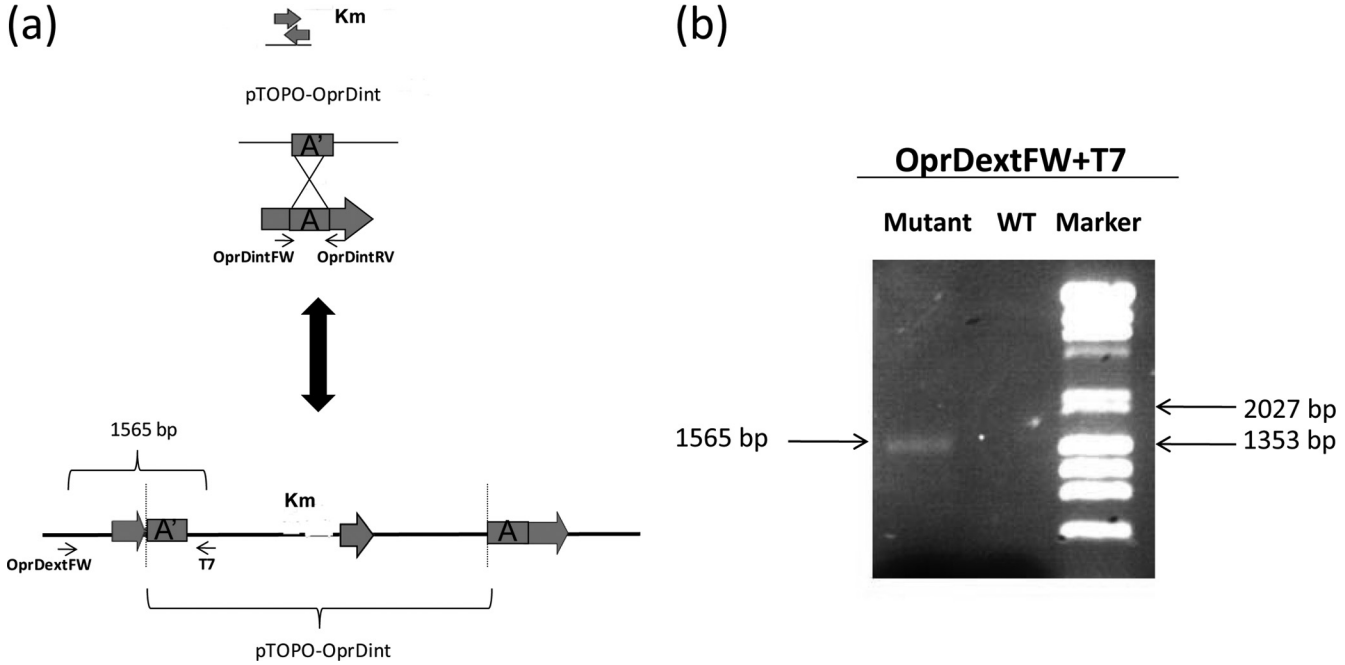

FIG 1 *oprD* homologue gene disruption. (a) Schematic representation of the strategy used to construct the *oprD*-like mutant by gene disruption. The oligonucleotides used are represented by small arrows. The boxes labeled A and A′ represent the original and cloned internal fragments of the *oprD* homologue gene, respectively. See Materials and Methods for details. (b) *oprD* homologue *A. baumannii* mutant generated by gene disruption. A PCR product of 1,565 bp (amplified with primers OprDextFW and T7) was sequenced to confirm *oprD* homologue gene disruption. A mixture of HindIII-digested lambda DNA and HaeIII-digested ϕX174 DNA (Finnzymes) was used as a molecular size marker. The genomic DNA of the wild-type strain (WT) was used as a negative control. The lengths of the PCR products and of some molecular size marker fragments are indicated.

Aldrich, France) dissolved in 1 M KCl–20 mM HEPES. The solutions were adjusted to pH 7 before their addition to the measurement cell.

RESULTS AND DISCUSSION

In *P. aeruginosa*, the OprD porin plays a significant role in the uptake of basic amino acids and carbapenems (23, 59), and its loss by resistant isolates is an important determinant of carbapenem resistance and is clinically relevant (43, 51). The identification of an OprD homologue, lost by several carbapenem-resistant *A. baumannii* strains, has suggested that this protein might function as a specific porin involved in carbapenem diffusion (16, 31, 33). For the first time, we investigated its potential function. We first compared the antibiotic sensitivity of an *A. baumannii* Δ*oprD* mutant to that of the wild-type strain.

Loss of the OprD homologue protein in *A. baumannii* OM: impact on antibiotic susceptibilities. The gene disruption method was used to inactivate the *A. baumannii oprD* homologue. It was carried out by cloning an internal fragment of the *oprD* homologue into a suicide plasmid (Fig. 1a). After the transformation of *A. baumannii* ATCC 17978 with the recombinant plasmid and selection on appropriate media, the *A. baumannii* Δ*oprD* homologue mutant was obtained (Fig. 1b). To determine whether a lack of the protein affects antimicrobial susceptibilities, we tested the Δ*oprD* homologue mutant's response to antibiotics of different classes. Compared to the wild-type parent, the Δ*oprD* homologue mutant did not present differences in the MICs of any antibiotics tested, i.e., imipenem, meropenem, ceftazidime, ciprofloxacin, and colistin (Table 1). Contradictorily, a previous study (26) revealed a 4-fold increase in the imipenem and meropenem MICs (from 4 to 16 μg/ml and from 0.5 to 2 μg/ml, respectively) for an OprD-defective *P. aeruginosa* strain and no differences in the MICs of β-lactams and quinolones. Thus, the present data suggest that the *A. baumannii* OprD homologue is not related to resistance to carbapenems. A compensatory mechanism, i.e., the increased expression of another porin, to explain the unmodified MICs of this Δ*oprD* homologue mutant could not be ruled out, however. Therefore, further functional characterizations of this protein were performed.

OprD family in *A. baumannii*. For bacteria like *P. aeruginosa* and *A. baumannii* that do not possess general diffusion porins and so present remarkably low OM permeability (23, 52), specific porins are usually required to facilitate diffusion through the OM in nutrient-limited environments. Owing to its large OprD protein family, *P. aeruginosa* is able to grow on a large array of metabolites while excluding other potentially harmful compounds. Different research groups have deciphered substrates of the different OprD family members (2, 10, 28, 36, 55, 58).

To further examine the function of the OprD homologue, we

TABLE 1 MICs for the *A. baumannii* wild-type strain and the Δ*oprD* homologue mutant

Drug	MIC (μg/ml)	
	ATCC 17978	Δ*oprD* mutant
Imipenem	0.25–0.38	0.25–0.38
Meropenem	0.5	0.5
Colistin	0.5	0.5
Ceftazidime	6	6
Ciprofloxacin	0.25	0.25

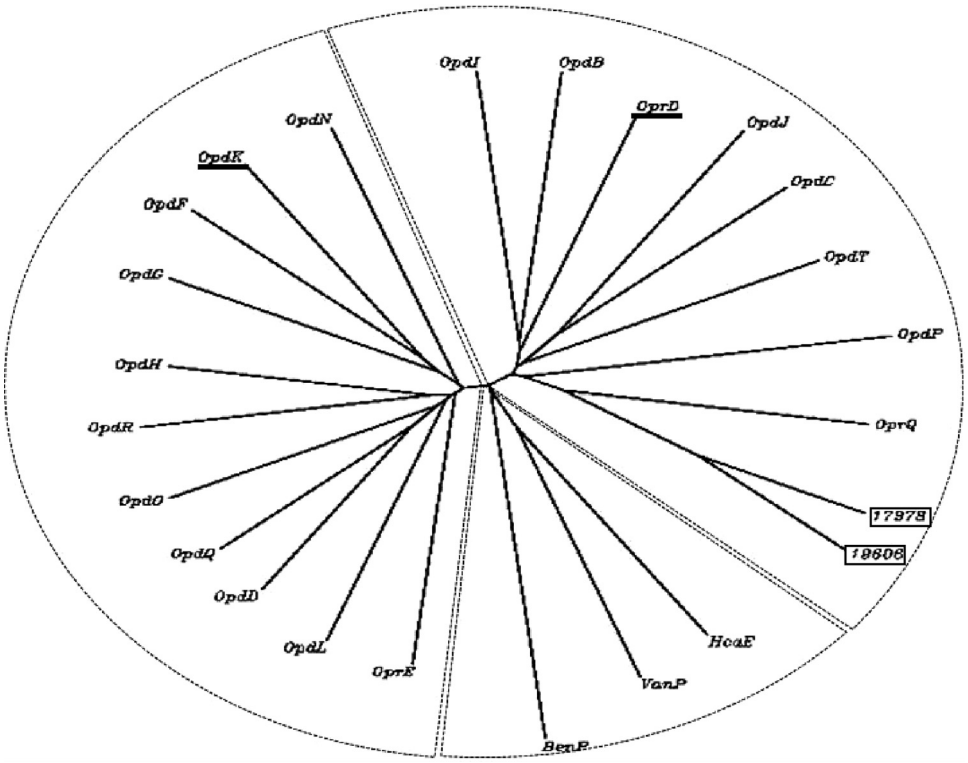

FIG 2 Phylogenetic unrooted dendrogram of OprD superfamily members obtained according to the neighbor-joining method at the http://www.genome.jp/tools/clustalw/ site. The genes for the three *A. baumannii* proteins BenP (YP_001084897.1), VanP (YP_001084148.1), and HcaE (YP_001084139) form a distinct subgroup. The number 17978 stands for the OprD homologue protein of *A. baumannii* ATCC 17978 (YP_001083280.1). The number 19606 stands for the OprD homologue protein of *A. baumannii* ATCC 19606 (ZP_05828632.1).

undertook amino acid sequence analyses and comparisons. The OprD homologue sequence from ATCC 17978 was first blasted against the 14 *A. baumannii* genomes at the Genoscope site (http://www.genoscope.cns.fr/agc/microscope/mage/). All sequences showed at least 80% identity (data not shown). Thirteen of them presented 98 to 100% identity with the ATCC 19606 OprD homologue. This indicates that the sequences of OprD homologues constitute a very homogeneous group, unlike what was reported for the OMP involved in imipenem diffusion, i.e., the CarO porin (9). Thus, modulation of substrate specificity in OprD homologues, as reported for CarO proteins, would be rather improbable (9).

Via this approach, we also showed that the *A. baumannii* 17978 genome (56) encoded three other members of the OprD superfamily, i.e., HcaE, BenP, and VanP, showing 45.3, 38.4, and 42.3% similarity to *P. aeruginosa* OprD, respectively. These *A. baumannii* sequences were compared with those of the 19 *P. aeruginosa* OprD superfamily members according to the neighbor-joining method as an unrooted tree (Fig. 2). This phylogenetic analysis allowed us to identify three distinct subgroups. The first group is defined by three of the *A. baumannii* sequences (BenP, VanP, and HcaE) on which few data are available (10, 36, 55). The other two subgroups (Fig. 2) have already been described by Tamber et al. (58) as the *P. aeruginosa* OprD and OpdK subgroups. Members of the OprD subfamily would take up the amino acids and related molecules, whereas OpdK homologues would be responsible for the uptake of a diverse array of organic acids (58). Interestingly, the *A. baumannii* OprD homologue sequence was effectively included in the OprD subgroup, being closer to the *P. aeruginosa* OprQ than to the OprD sequence (59.2 and 49.6% similarity, respectively). From these analyses, we consequently examined the hypothesis that the OprD homologue may function as an OprQ-like protein.

Modulation of OprD homologue protein expression. Although the exact role of OprQ in *P. aeruginosa* nutrient uptake is still unknown (58), some studies have suggested that this protein would be required for *Pseudomonas* survival under stress conditions, including iron-depleted or low-oxygen environments (2, 28). We investigated the expression of the OprD homologue in *A. baumannii* ATCC 19606 by semiquantitative RT-PCR experiments. No transcriptional alteration was observed under any of the conditions tested (data not shown). To further evaluate the specificity of this porin for Fe^{3+}, we planned a functional analysis by reconstitution in planar lipid bilayers, which required preliminary overexpression and purification of the OprD homologue.

Expression and purification of OprD homologue protein. The gene encoding the N-terminally His_6-tagged recombinant protein, i.e., the *oprD* homologue from ATCC 19606, was cloned into the pETSIG vector and transformed into *E. coli* BL21(DE3) pLysS (54). Overexpression was induced by the addition of IPTG. Owing to the presence of the N-terminal OmpA signal peptide fused to the mature protein, the recombinant protein could be directed to the OM of *E. coli*, generating an active protein. As already mentioned by Biswas et al. (6), the rOprD homologue could not be solubilized directly from the cell envelope (whatever the detergent used) and preseparation of the OM and IM by the *N*-lauroylsarcosine method was necessary (22). Solubilization of the OprD homologue from the OM pellet was finally achieved

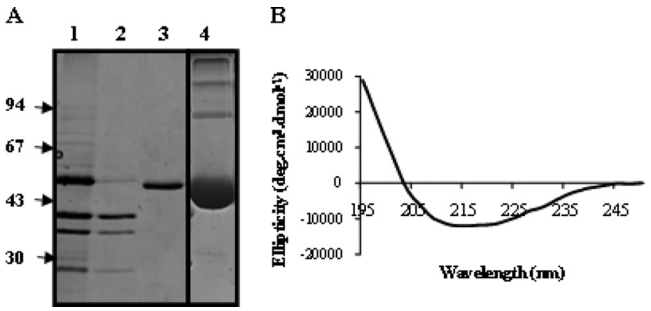

FIG 3 Purification of OprD homologue recombinant protein. (A) SDS-PAGE purification with a 7 to 12% discontinuous gel system. All samples were heated for 10 min at 100°C before loading. Lane 1, fraction of OprD homologue solubilized in 0.3% LDAO. Lane 2, flowthrough fraction after injection through a Ni-NTA column. Lane 3, fraction after elution with 400 mM imidazole. Lane 4, concentrated rOprD homologue. The values to the left are molecular sizes in kilodaltons. (B) Circular dichroism spectrum of the OprD homologue.

with 0.3% LDAO as shown in Fig. 3A (lane 1). Purification was performed by affinity chromatography using Ni-nitrilotriacetic acid (NTA) resin and allowed the recovery of the recombinant protein at 0.2 to 1.5 mg/ml (Fig. 3A, lanes 2 to 4). The OprD homologue migrated as a monomer in SDS-PAGE (Fig. 3A, lane 3). However, more concentrated fractions reveal dimers and trimers (Fig. 3A, lane 4). This observation agrees with the formation of labile trimers that dissociated in SDS, as described for the *P. aeruginosa* OprD and OpdK proteins (5, 61). Circular dichroism analysis (Fig. 3B) confirmed that the protein retained its native β-sheet secondary structure, as the spectrum presents a minimum of ellipticity between 210 and 220 nm, a characteristic of OMPs such as porins (5, 6, 17). Lastly, the His_6 tag was cleaved by thrombin digestion (9). N-terminal sequencing of this tagless rOprD homologue sample gave the sequence GSSEQSEAKG. SEQSEAKG corresponded to the first amino acids of the mature sequence (16) and demonstrated that the rOprD homologue was obtained free of the tag and ready for functional analyses.

Functional analyses. To characterize the ionophore properties of the rOprD homologue, the purified protein was reincorporated into DPhPC planar lipid bilayers. After addition of the protein at 10^{-9} M to the *cis* compartment of the measurement cell and application of the voltage, discrete current fluctuations were induced corresponding to a single-channel conductance value of 28 ± 8 pS in 1 M KCl. During reconstitution experiments, a higher conductance value of 95 ± 8 pS was also observed, which could correspond to the insertion of trimers into the membrane. Figure 4A shows the opening and closure of a single channel from an already open state corresponding to a possible trimer. These conductance

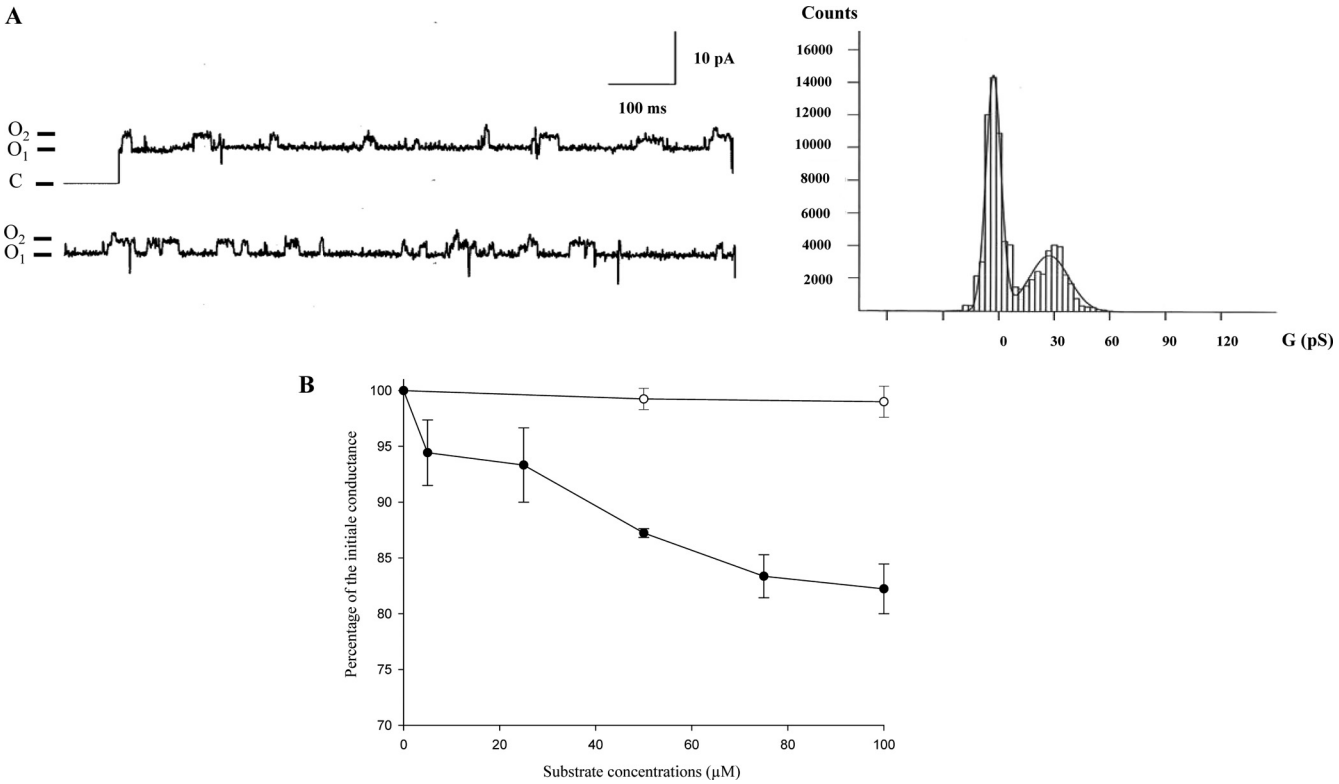

FIG 4 Ionophore properties of the *A. baumannii* rOprD homologue. (A) rOprD homologue reconstitution in a DPhPC bilayer. C, closed state; O_1, first open state; O_2, second open state. The recordings shown were made at an applied voltage of 70 mV in 1 M KCl–20 mM HEPES, pH 7.4. Digitization rate, 3,000 Hz; filter, 300 Hz. The inset amplitude histogram with Gaussian fitting was used to determine the conductance value of the monomeric state (28 ± 8 pS). (B) Macroscopic conductance inhibition experiments to examine the binding of different substrates. The porin at 10^{-8} M was added to the measurement compartment. When the final conductance was stabilized (reincorporation of about 200 channels), aliquots of imipenem (open circles) or Fe^{3+} (filled circles) were added to both sides of the membrane to reach the concentrations shown on the abscissa (in μM). The decrease in conductance due to the binding of the substrate and the blocking of ion flux through the channel was monitored, and the decreased conductance is expressed as a percentage of the initial conductance. Each point represents the average of three to five measurements.

values are in good agreement with those reported for other OprD family members like *P. aeruginosa* OprD itself (20 pS in 1 M KCl [6, 27]) or *P. fluorescens* OprQ (30 pS in 1 M NaCl [28]).

Specificity measurements were performed by macroscopic conductance inhibition experiments (3, 9, 27). Briefly, after the addition of the rOprD homologue at 10^{-8} M to the measurement cells, the resulting macroscopic conductance increased (due to the reincorporation of about 200 channels in the bilayer) before stabilizing. Substrates were added on both sides of the bilayer, and the decrease in conductance induced by the binding of the substrate in the channel and the impeded ionic current was recorded. Several substrates were tested according to the hypothesis that the protein could be either an OprD-like or an OprQ-like channel. As expected, the OprD homologue did not present any specificity for basic amino acids like histidine, arginine, or ornithine or for carbapenems (imipenem and meropenem). However, as shown in Fig. 4B, increasing concentrations of Fe^{3+} induced channel closure (which was not observed when Al^{3+} was tested), and a binding constant, $K = 4{,}911\ M^{-1}$, can be calculated accordingly (3). As *P. aeruginosa* OprQ expression was also shown to be slightly induced under low-Mg^{2+} conditions (2), we tested the specificity of the OprD homologue to this ion. The addition of this compound to the measurement cells resulted in a clear but much smaller decrease in macroscopic conductance (reaching 16% at 20 mM), highlighting the low specificity of the OprD homologue for this ion.

These data confirm that the OprD homologue protein in *A. baumannii* may possess a function similar to that of *P. aeruginosa* OprQ. The channel open state could facilitate the capture and diffusion of essential Fe^{3+} and Mg^{2+} when adaptation to magnesium- and/or iron-depleted environments is required.

Concluding remarks. Collectively, the data presented here are evidence (*in vivo* and *in vitro*) that the *A. baumannii* OprD homologue is not involved in specific antibiotic diffusion and would consequently not contribute to carbapenem resistance in this species. This channel might, rather, have functions similar to those of the *Pseudomonas* OprQ protein, offering specific binding sites for iron and magnesium ions and allowing *A. baumannii* to adapt to stress conditions. As reported for recent proteomic studies, the OprD homologue, which is overexpressed during sessile growth mode, would promote *A. baumannii* biofilm formation (8, 35). It is tempting to consider that this protein might be involved in cell adhesion, as its OprQ counterparts are in *P. aeruginosa* and *P. fluorescens* binding to fibronectin (2, 49). Associated with its particular affinity for iron, these features could contribute to the involvement of the OprD homologue (which could now be called the OprQ-like protein) in the host colonization process and make it a potential and important virulence factor of *A baumannii*.

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